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ON THE DEVELOPMENT OF THE SEA-STAR, *ASTROPECTEN LATESPINOSUS* MEISSNER

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There have been several reports on the development of sea-stars belonging to the genus *Astropecten*. Newth (1925) reported on the early development in *Astropecten irregularis*, and Hörstadius (1939) gave a detailed description of the entire process of development in *Astropecten aranciacus*. Further, Mortensen (1921, 1937) outlined the form of bipinnariae of *Astropecten scoparius, Astropecten polyacanthus* and *Astropecten velitaris*. It has been known that all of these sea-stars form typical bipinnariae. The majority of the species of *Astropecten* undergoes metamorphosis without passing through the brachiolaria stage.

The present study was initiated to investigate the development of *Astropecten latespinosus,* which is one of the most common sea-stars in Japan. It becomes evident in the present study that the development of this species is somewhat different from that of the other species of *Astropecten*, in having a larva of barrel form and in undergoing metamorphosis very early.

In the present paper, the entire process of development in *Astropecten latespinosus* is described, especially the external morphology and the skeletal system. Developmental processes in the internal structure will be reported later.

Materials and Methods

Adults of the sea-star, *Astropecten latespinosus* Meissner were collected on the coast of Toyama Bay, Sea of Japan, during the presumed breeding season, from the end of June through July in 1972 and 1973.

By treatment with 1-methyladenine of the ovaries which were removed from the females according to Kanatani (1969), fertilizable ova were obtained. Dilute sperm suspension was prepared from small pieces of mature testes and added to the petri-dish containing the ova. Embryos and juveniles were reared in glass vats at laboratory temperature of 25 ± 2° C.

Observations were made with a dissecting microscope. Measurement of living embryos was performed with an ocular micrometer. Specimens were fixed at appropriate intervals with Bouin's solution or 10% formalin in sea water for later detailed observations of sectioned material. For microscopic examination of the skeletal system larvae were fixed in 70% alcohol, then macerated in a 10% aqueous solution of potassium hydroxide.

Observations

The mature ova are spherical, semitranslucent, brownish cream in color, and are enclosed in a jelly layer about 10 μ thick. They measure from 250 μ to 400 μ in diameter with a mean of about 300 μ. They are heavier than sea water.

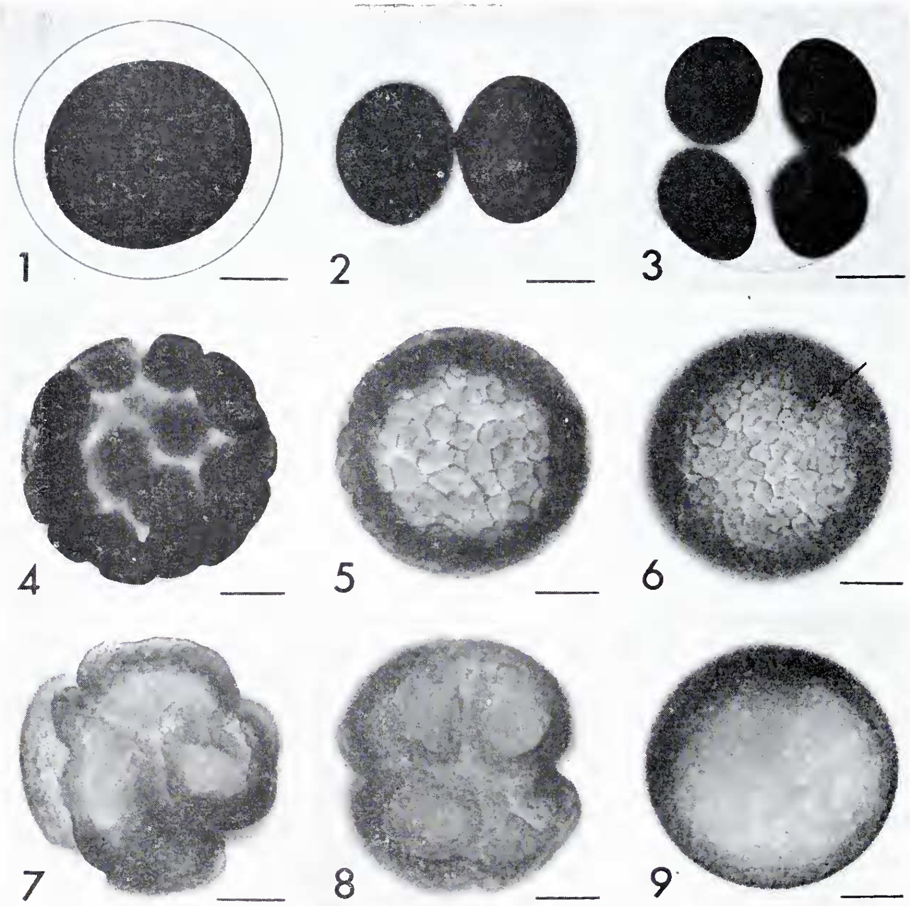

FIGURE 1. Fertilized egg with elevated fertilization membrane; scale = 100 μ.
FIGURE 2. Two-cell stage; scale = 100 μ.
FIGURE 3. Eight-cell stage; scale = 100 μ.
FIGURE 4. Thirty two-cell stage; scale = 100 μ.
FIGURE 5. Early coeloblastula; scale = 100 μ.
FIGURE 6. Beginning of wrinkled blastula stage. Arrow points egression tract; scale = 100 μ.
FIGURE 7. Wrinkled blastula with conspicuous egression tracts, 2 hours later than Figure 6; scale = 100 μ.
FIGURE 8. Later wrinkled blastula stage; scale = 100 μ.
FIGURE 9. Blastula just after resuming smooth surface; scale = 100 μ.

About two or three minutes after insemination, the fertilization membrane began to elevate, and 60 minutes thereafter the process was completed, with a perivitelline space 50 μ height (Fig. 1). The cleavage is of holoblastic, radial type. Seventy minutes after insemination, the first cleavage occurred through the animal-vegetal axis (Fig. 2). The embryos were in the 8-cell stage 120 minutes after insemination (Fig. 3), and in the 32-cell stage 150 minutes after insemination (Fig. 4). They developed into early coeloblastulae 3.5 hours after insemination (Fig. 5). Four hours after insemination, streaks (egression tracts) appeared on the surface of the blastula and then the embryos were entering the wrinkled blastula stage (Fig. 6). The egression tracts gradually increased in number and size, and embryos reached the most wrinkled stage 2 hours after the beginning of wrinkling (or 6 hours after insemination; see Fig. 7). Duration and magnitude of wrinkling, however, seemed to be somewhat different among individuals. Then, the egression tracts began to decrease in number and complexity (Fig. 8). Seven and one half hours after insemination, the surface of the blastulae resumed smoothness (Fig. 9). Eight hours after insemination gastrulation took place at the vegetal pole (Fig. 10). As far as can be ascertained from the present observations, the invagination for gastrulation apparently had no connection with egression tracts for wrinkling. Nine hours after insemination, early gastrulae began to rotate within the fertilization membrane.

One hour thereafter (10 hours after insemination) they hatched as free-swimming larvae. Two or three hours after hatching (12–13 hours after insemination), the blind end of the archenteron expanded, and then mesenchymal cells were set free into the blastocoel (Fig. 11). Ten hours after hatching (20 hours after insemination), a pair of the rudimental coelomic pouches was recognized at both sides of the tip of the archenteron. The gastrulae gradually elongated along the archenteric axis, and by 20 hours after insemination they had reached a length of 600 μ (Fig. 12). A few hours later, the archenteron seemed to be differentiated into stomach and intestine (Fig. 13). However, the details of the internal organogenesis could not be observed from outside due to the opacity of the larvae.

Thirty hours after insemination, the larvae showed a barrel form as shown in Figure 14. The length and the width of larvae at this stage were 700 μ and 350 μ, respectively, and the coelomic pouches were separated from the archenteron. The posterior wall of the larva was thicker than the anterior wall. Then the posterior end began to become rounded. Forty-five hours after insemination, 5 lobes of the hydrocoel became evident (Fig. 15). The posterior part of a larva corresponds to the future body of the sea-star after metamorphosis, and might be called a larval disk. On the other hand the anterior part, which will be reduced in size during metamorphosis, corresponds to the stalk of the brachiolaria larva. Two or three hours thereafter (47–48 hours after insemination), the distinction of these parts became more evident (Fig. 16). At that time both coelomic pouches extended so as to contact with each other by their anterior ends. Fifty hours after insemination, the skeletal plates began to be formed as spicules on the future aboral side of the larval disk (Fig. 28a). These spicules correspond to 1 madreporic and 5 terminal plates (Fig. 28b). Fifty-five hours after insemination, 10 processes arose on the peripheral part of the larval disk (Fig. 17). The hydrocoel was recognized in the future oral side of the larval disk (Fig. 18). At the opposite side of the larval disk, a central plate appeared as a spicule (Figs. 19 and 20). There were several spines on the terminal plate. A little while after, 5 pairs of rudimental oral plates appeared on the future oral side of the larval disk (Fig. 21). About 70 hours after insemination, the larvae moved slowly while the top of their future aboral side was in contact with the surface of the substratum (Fig. 22). On the fourth day of development (about 75 hours after insemination),

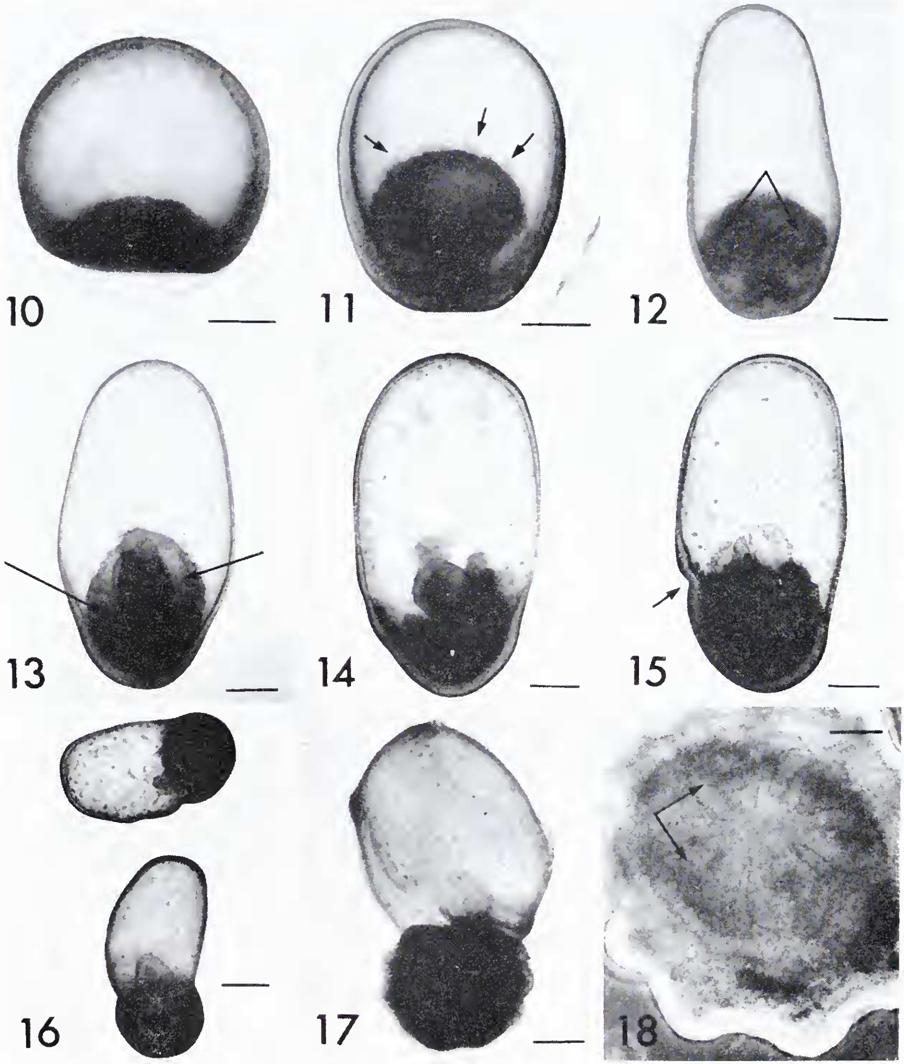

FIGURE 10. Early gastrula, just after beginning of invagination; scale = 100 μ.

FIGURE 11. Gastrula little later than that shown in Figure 10. Note expanded top of archenteron and liberation of mesenchymal cells (arrows); scale = 100 μ.

FIGURE 12. Gastrula, 20 hours after insemination. Arrows show rudiments of coelomic pouch; scale = 100 μ.

FIGURE 13. Gastrula; 5 hours later than that shown in Figure 12. Each coelomic pouch (arrows) elongates near the posterior end of larva; scale = 100 μ.

FIGURE 14. Larva in a barrel form, lateral view. Each coelomic pouch is separated from the archenteron; scale = 100 μ.

FIGURE 15. Metamorphosing larva, 45 hours after insemination. Note constriction (arrow) between disk and stalk; scale = 100 μ.

FIGURE 16. Two larvae showing opposite side to each other, one (upper) shows future aboral side and the other future oral side; 2 or 3 hours later than that shown in Figure 15, scale = 200 μ.

larvae attached to the substratum with the anterior portions of their stalks (Fig. 23).

Metamorphosis proceeded rapidly after attachment. The stalk began to degenerate (Fig. 24). The rudiments of two pairs of tube-feet and of a single terminal tentacle appeared on each lobe of the hydrocoel. At the same time, the formation of the skeletal system progressed further. Several spicules which correspond to the radial and interradial plates appeared around the central plate on the future aboral side (Figs. 30a and 30b). On the reverse side of the disk, the rudimental ambulacral plates appeared (Figs. 31a and 31b). The juvenile shown in Figure 25 was photographed 20 hours after attachment (or about 95 hours after insemination). The reduced stalk still remained in this stage. About 5 days after insemination the stalk was completely absorbed, and the opening of the mouth was recognized at the center of the oral side. Juveniles immediately after metamorphosis were white in color and 500 μ in diameter (Fig. 26). They had 2 long spines, each 10 μ in length, at the top of each of 5 arms. The skeletal system in this stage is shown in Figures 32a, 32b and 32c. Later an eye-spot appeared on the basal portion of each terminal tentacle (Fig. 33). At this stage, the tube-feet were functional having suckers at their tips, enabling juveniles to move about. Twenty-five days after insemination, each arm was distinguishable from the disk. At that time, a central plate and other dorsal plates developed as shown in Figure 27.

Although about 300 juveniles were alive in the laboratory for about 40 days after insemination, they did not show further differentiation and eventually died. *Artemia*-larvae or clam meat were not acceptable as food. The skeletal system of the juvenile, which is 300 μ in R (the distance from the center of the disk to the tip of the arm) and 200 μ in r (the distance from the center of the disk to the middle of the interradial margin), is given in Figures 34a, 34b and 34c. As shown in the figures, each arm has a terminal plate with about 10 spines. Dorsal plates, with 1 spine at the center, lie compactly on the aboral side of the disk. A madreporic plate was recognizable on one interradius. Five pairs of oral and ambulacral plates developed on the oral side.

Discussion

In the present study, it was found that the development of *Astropecten latespinosus* is quite different from that of the other sea-stars, in the following three points: (1) there is a peculiar larval form, (2) metamorphosis takes place while the larva is pelagic; there is no brachiolaria stage; (3) the larval life is very short.

There has been no previous report of the swimming larvae of asteroids having the morphology described here. They are barrel-shaped, somewhat resembling the doliolaria larva but lacking in definite ciliary bands. Metamorphosis is initiated at the posterior part of the body while the larva is pelagic; fixing disk and brachiolar arms are not formed, although these are usually found during metamorphosis in sea-stars. The larva completes metamorphosis without feeding, as reported

FIGURE 17. Larva, 55 hours after insemination; scale = 100 μ.

FIGURE 18. Larval disk showing future oral side. Same stage as shown in Figure 17. Arrows point primordial lobes of hydrocoel; scale = 50 μ.

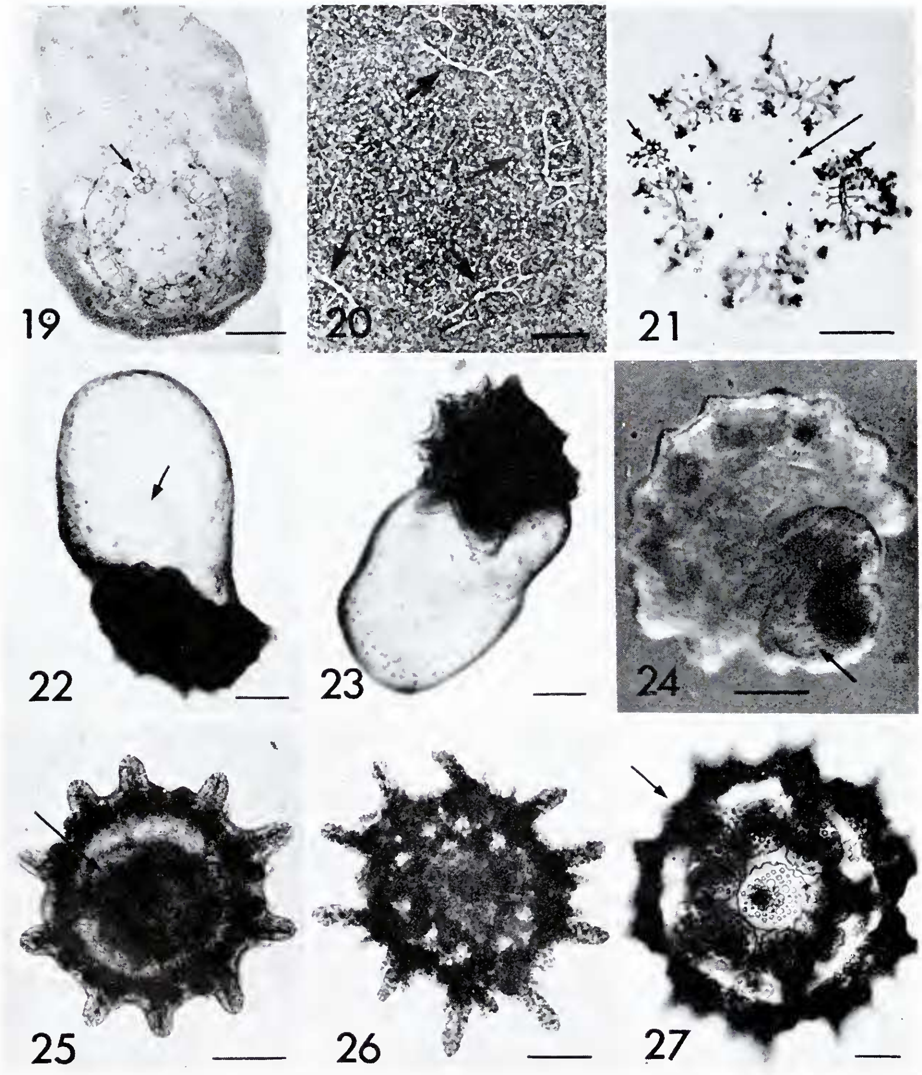

FIGURE 19. Opposite side (future aboral side) of the same larva as in Figure 18, showing rudimental skeletal plates. Arrow indicates madreporic plate; scale = 100 μ.

FIGURE 20. Enlarged picture of a part of the disk shown in Figure 19, showing details of terminal plates indicated by arrows (phase contrast microscopy); scale = 50 μ.

FIGURE 21. Skeletal system of the larva, slightly later than that shown in Figure 19; prepared by the treatment of KOH solution. Short and long arrows point to madreporic plate and oral plate, respectively; scale = 100 μ.

FIGURE 22. Larva just before attachment, lateral view. Arrow shows the anterior coelom; scale = 100 μ.

FIGURE 23. Four day-old larva, just after attachment to the substratum; scale = 100 μ.

FIGURE 24. Attached larva, future oral side, pictured through the glass bottom. Arrow points degenerating stalk; scale = 100 μ.

for some species such as *Crossaster papposus, Leptasterias ochotensis similispinis* or *Ceratonardoa semiregularis* (Gemmill, 1920; Kubo, 1951; Hayashi and Komatsu, 1971).

Development of the sea-stars has been generally divided into two types, the indirect and the direct, mainly based on what type of larva appears. In indirect development, the embryo develops into a brachiolaria after passing through the bipinnaria stage; it attaches to the substratum by fixing disk or brachiolar arms before the completion of metamorphosis. This type of development has been reported in *Asterias rubens, Porania pulvillus, Asterias amurensis* and *Acanthaster planci* (Gemmill, 1914, 1915; Dan, 1957; Yamaguchi, 1971). The second type, direct development, passes through only the brachiolaria stage; a bipinnaria stage is entirely lacking. *Asterina gibbosa, Henricia sanguinolenta, Solaster endeca, Echinaster echinophorus* are among those having direct development (MacBride, 1896; Masterman, 1902; Gemmill, 1912; Atwood, 1973). It may be an object of argument to classify the type of development of the present species, because it does not form either typical bipinnaria or brachiolaria. The distinction, however, between those two types has not given very clearly. An attempt has been made by some workers to give a precise definition for two types of development. Hyman (1955) described the direct type as a kind of development lacking in free larvae. However, one may regard the swimming brachiolariae found in *Crossaster papposus* or *Certonardoa semiregularis* as free larvae in spite of the fact that they undergo direct development. It was proposed by Chia (1968) that development with a larva having a functional larval gut should be called the indirect type and those without a functional gut the direct type. This distinction may be useful in dividing all asteroid larvae into two types. The development of *Astropecten latespinosus*, therefore, is of the direct type according to Chia's classification.

It appears that there is an intimate relationship between the size of the egg and the type of development. Eggs having direct development are generally larger than those with indirect development. The largest egg having indirect development, is that of *Astropecten scoparius* which is 230 μ in diameter (Komatsu, 1973). On the other hand, eggs characterized by direct development are more than twice the size of those with indirect development (see Hayashi, 1972); the smallest so far reported is about 500 μ in *Asterina gibbosa* (MacBride, 1896). The majority of the sea-stars undergoing direct development take more than 30 days to complete metamorphosis after fertilization, except *Echinaster echinophorus* (Atwood, 1973) which takes about 14 days to complete metamorphosis. The egg of the present species is about 300 μ in diameter, and is thus being very small among eggs with direct development. The present species completes metamorphosis within

FIGURE 25. More advanced stage than that shown in Figure 24, future oral side. Note the bulges for 2 pairs of tube-feet and ten long spines on the terminal plates. Arrow shows reduced stalk; scale = 100 μ.

FIGURE 26. Juvenile immediately after the completion of metamorphosis, oral view; scale = 100 μ.

FIGURE 27. Aboral skeletal plates of a juvenile, 25 days after insemination. Note large central plate in the center and radial and interradial plates around it. Upper left (arrow) shows madreporic plate. Prepared by the treatment of KOH solution; scale = 50 μ.

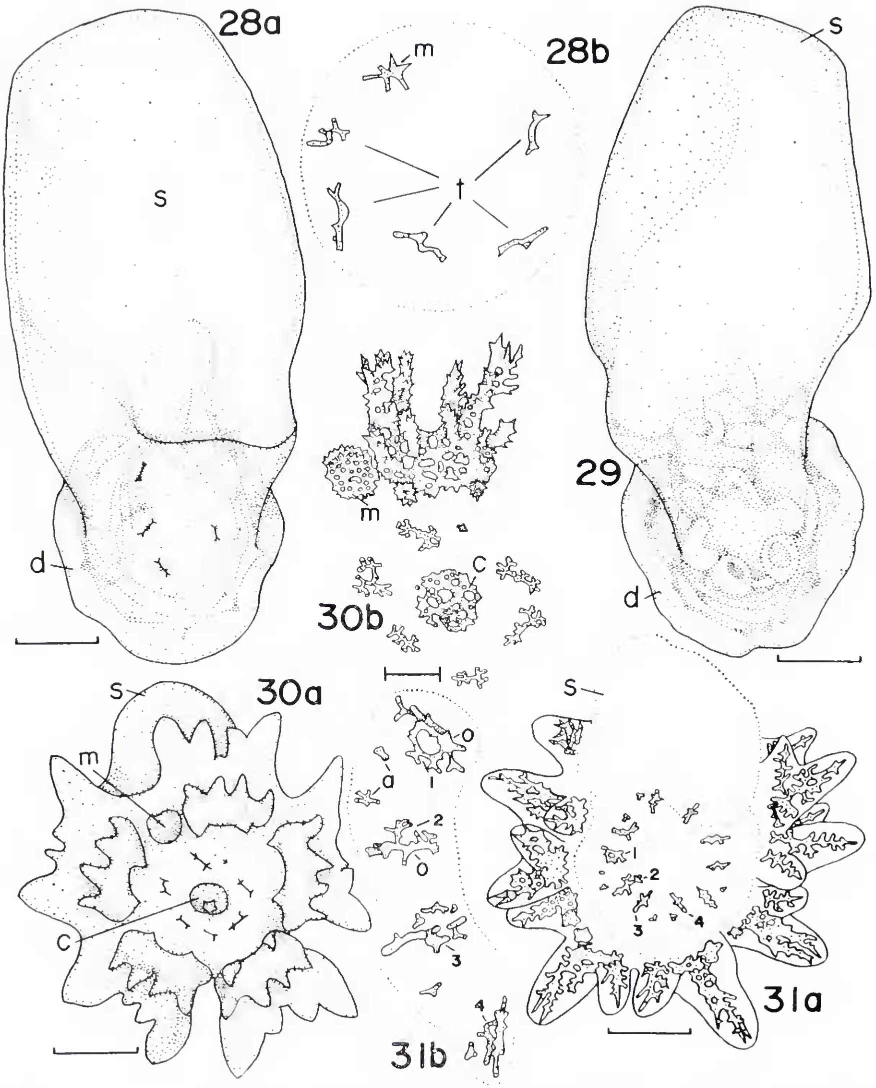

FIGURE 28a. Free-swimming larva, 50 hours after insemination, showing future aboral side of disk; d, disk; s, stalk; scale = 100 μ.

FIGURE 28b. Primordia of the skeletal plate, same stage as shown in Figure 28a; m, madreporic plate; t, terminal plate.

FIGURE 29. Opposite side (future oral side) of that shown in Figure 28a; d, disk; s, stalk; scale = 100 μ.

FIGURE 30a. Skeletal system of attached larva, future aboral side; c, central plate; m, madreporic plate; s, stalk; scale = 100 μ.

FIGURE 30b. Detailed sketch of plates, same specimen as shown in Figure 30a; c, central plate; m, madreporic plate; scale = 25 μ.

FIGURE 31a. Skeletal system, opposite side of that shown in Figure 30a; s, stalk; scale = 100 μ.

FIGURE 31b. Detailed sketch of oral and ambulacral plates, same specimen as shown in Figure 31a. Numbers (1, 2, 3 and 4) correspond to the numbers (1, 2, 3 and 4) of Figure 31a; a, ambulacral plate; o, oral plate; scale = 25 μ.

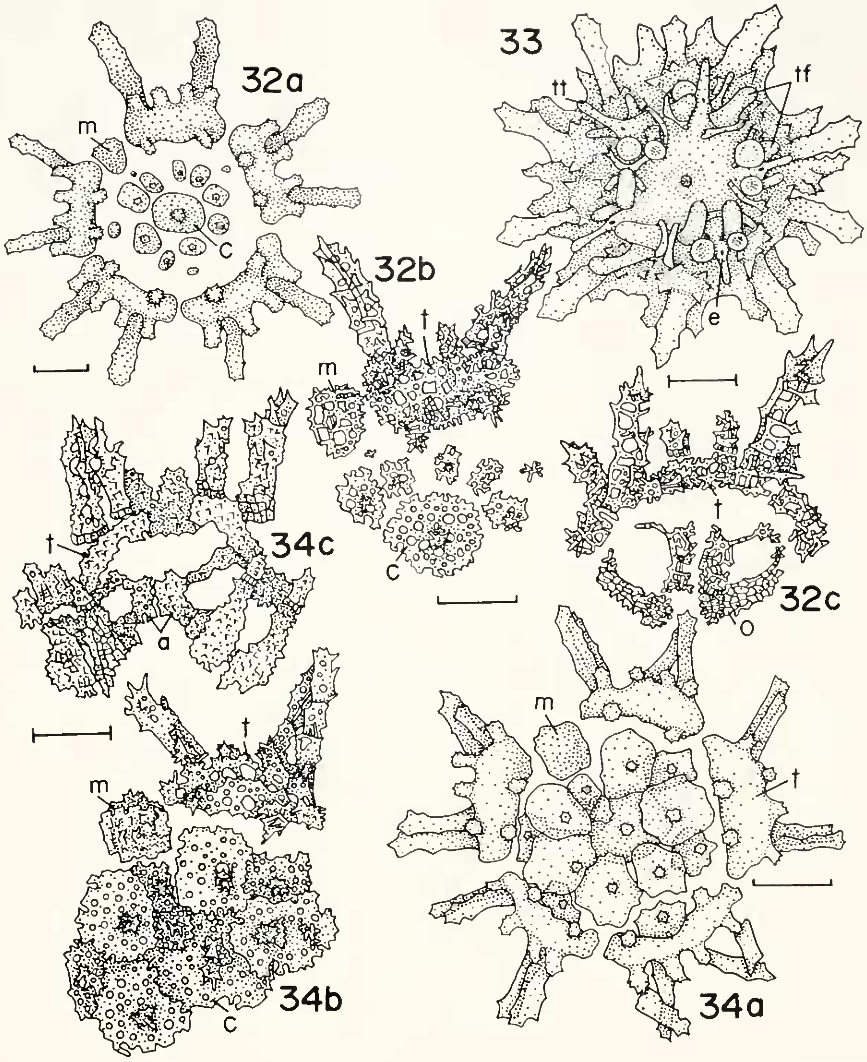

FIGURE 32a. Aboral skeletal system of the juvenile, same stage as shown in Figure 26; m, madreporic plate; c, central plate; scale = 100 μ.

FIGURE 32b. Detailed sketch of plates, same specimen as shown in Figure 32a; c, central plate; m, madreporic plate; t, terminal plate; scale = 75 μ.

FIGURE 32c. Detailed sketch of plates on oral side, same specimen as shown in Figure 32b; o, oral plate; t, terminal plate; scale = 50 μ.

FIGURE 33. Oral side of the juvenile, little later than that shown in Figure 26; e, eye-spot; tf, tube-foot; tt, terminal tentacle; scale = 50 μ.

FIGURE 34a. Aboral skeletal system of 40 day-old juvenile; m, madreporic plate; t, terminal plate; scale = 100 μ.

FIGURE 34b. Detailed sketch of plates, same specimen as shown in Figure 34a; c, central plate; m, madreporic plate; t, terminal plate; scale = 75 μ.

FIGURE 34c. Detailed sketch of plates, oral side of the same specimen as shown in Figure 34; a, ambulacral plate; t, terminal plate; scale = 75 μ.

7 days after insemination and this speedy development may be related to the small size of the egg.

It was reported for all species of the genus *Astropecten* thus so far studied that metamorphosis takes place while larvae are pelagic as typical bipinnaria (Mortensen, 1921, 1937; Hörstadius, 1939). As noted above, *Astropecten latespinosus* also undergoes metamorphosis while the larvae are pelagic, although it does not form bipinnaria, but rather a barrel-shaped larva. The present species thus may show a much abbreviated process of development in comparison with the other species of *Astropecten*; presumably this is due to the lecithotrophic nature of the egg.

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Summary

1. The entire process of development in the sea-star, *Astropecten latespinosus*, is reported, especially with regard here to the external morphology and the skeletal system.

2. The eggs are medium-sized, about 300 μ in diameter. They develop into free-swimming larvae through a wrinkled blastula stage by holoblastic, radial cleavage.

3. The free-swimming larva has a peculiar barrel shape, being neither bipinnaria nor brachiolaria. Such a larva has not previously been reported.

4. Metamorphosis takes place while the larva is pelagic, and there is no feeding at this stage. Five days after insemination, metamorphosis is completed and the resulting juveniles bear 2 pairs of tube-feet and a terminal tentacle in each arm.

5. The present observations are compared with those studied by other workers, and are discussed, with special reference to the type of development of sea-stars.

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